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PPARγ AND HIGH MOLECULAR WEIGHT ADIPONECTIN IN WHITE ADIPOSE TISSUE IN AN OBESITY MODEL

By

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Abstract

PPARγ AND HIGH MOLECULAR WEIGHT ADIPONECTIN IN WHITE ADIPOSE TISSUE IN AN OBESITY MODEL

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Adiponectin is a protein secreted from adipose (fat) tissue. It is secreted into the blood as either a trimer (low molecular weight), hexamer (middle molecular weight) or an 18-mer (high molecular weight). Adiponectin, particularly the high molecular weight (HMW) isoform, has been shown to increase insulin sensitivity, which is important for maintaining healthy blood glucose levels. Adiponectin levels have been shown to be decreased in individuals who are obese, and this may play a role in the diabetes which often accompanies obesity. PPARγ is a protein that is highly expressed in white adipose tissue and is responsible for regulating portions of metabolism such as glucose and fatty acid uptake and oxidation as well as proposed to be important in regulating adiponectin levels and the production of its high molecular weight isoform. This study examines the level of PPARγ and adiponectin isoforms in adipose tissue in a mouse model of obesity.

White adipose tissue samples were removed from mice fed a high-fat or low-fat diet for 6, 10, or 16 weeks. An enzyme-linked immunoassay (ELISA) was utilized for total adiponectin, HMW adiponectin, and PPARγ following homogenization and preparation of tissue sample. Levels of PPARγ, total adiponectin, and HMW adiponectin were statistically

evaluated over the time course of the experiment both within and between the treatment groups.

The level of PPARγ was not different over the course of low-fat feeding; however, the levels of PPARγ were significantly ($P<0.05$) increased in the 10 week high-fat fed mice compared to high-fat fed mice at 6 or 16 weeks and compared to the 10 week low-fat fed group. PPARγ was significantly ($P<0.05$) lower in the 16 week high-fat fed mice than in the low-fat fed 16 week group. There was no statistically significant alteration in either total or HMW adiponectin within or between treatment groups. Finally, the ratio of HMW adiponectin to total adiponectin was not significantly different within or between treatment groups.

Results of this study suggest that, while there were some changes in PPARγ with high-fat feeding they were not correlated with alterations in the adipose tissue levels of adiponectin. This indicates that there is not a tight connection between PPARγ and regulation of adiponectin levels in this model of obesity.

Introduction:

With obesity comes a plethora of personal and public health problems as well as economic concerns (Withrow and Alter, 2011). Obesity has been a growing problem over the past century as industrialization continues to increase throughout the world. Worldwide, there were 1.9 billion adults who were overweight and 650 million who were obese as of 2016 (http://www.who.int/en/news-room/fact-sheets/detail/obesity-and-overweight). Obesity can increase the risk of atherosclerosis and high blood pressure, and in turn, myocardial infarctions and strokes. Other risks of obesity include cancer, osteoarthritis, kidney and liver diseases, and diabetes (https://www.niddk.nih.gov/health-information/weight-management/adult-overweight-obesity/health-risks). Obesity has proven to be a daunting problem to solve, resulting in thousands of early deaths and billions of dollars spent in the US alone on healthcare and research related to obesity (https://www.cdc.gov/obesity/adult/causes.html). Obesity is a particular concern within Kentucky. As of 2015, 35.6% adults (18 years old or older) were overweight and 33.2% were obese, making Kentucky the state with the 5th highest rate of obesity (http://www.fitky.org/wp-content/uploads/sites/2/2015/12/Obesity-Fact-Sheet.pdf). Not only is obesity a public health and economic concern, it is a social issue as well as those of lower socioeconomic statuses tend to have higher rates of obesity (van der Klaauw et al, 2017).

Among the many effects of obesity is the harm that it does to the body's ability to control blood glucose levels. Blood glucose levels are a reflection of the changes in the input and output of glucose into blood plasma. Diabetes, a disease characterized by elevated blood glucose levels, is a major health problem in modern society and is caused by an inability of the body to control blood glucose levels. It is the 7th leading cause of death

and affects 30 million people in the United States (https://www.cdc.gov/diabetes/basics/quick-facts.html). Diabetes can result from either a complete or partial loss of insulin secretion from the pancreas (Type 1 or juvenile-onset), or a loss of target tissue sensitivity to the hormone (Type 2 or adult-onset). Associated with obesity is a decrease in insulin sensitivity, making it more difficult for the body to keep blood sugar from rising too high. Insulin helps the body regulate blood sugar by causing tissue uptake of glucose from the blood and by acting as an antagonist to the blood glucose increasing hormone glucagon. The frequent and possibly extreme hyperglycemia from diabetic conditions and loss of insulin sensitivity can cause kidney failure, loss of extremities, cataracts and blindness, gum infections, diabetic ketoacidosis, and diabetic coma (www.diabetes.org/living-with-diabetes/complications/).

One of the major regulators of insulin sensitivity is the secretory protein adiponectin. The first report of adiponectin was in 1995 under the name Acrp30 (Sherer et al, 1995), and since its discovery it has been well-studied due to its interactions with obesity and insulin sensitivity. Adiponectin is produced exclusively or predominantly by adipose cells. This hormone has been shown to target cells in a variety of tissues and, among other impacts, it improves the sensitivity of insulin in those tissues, which would cause a lower blood sugar when necessary, and helps combat previous insulin desensitization through diabetes or obesity. As a secretory protein, adiponectin is synthesized in association with the endoplasmic reticulum (Wang et al, 2008). An adiponectin monomer (see Figure 1) contains 247 amino acids in humans (Scherer et al, 1995) and consists of a C-terminal globular domain, a collagenous stalk, and an N-terminal signaling region (Ruan and Dong, 2016).

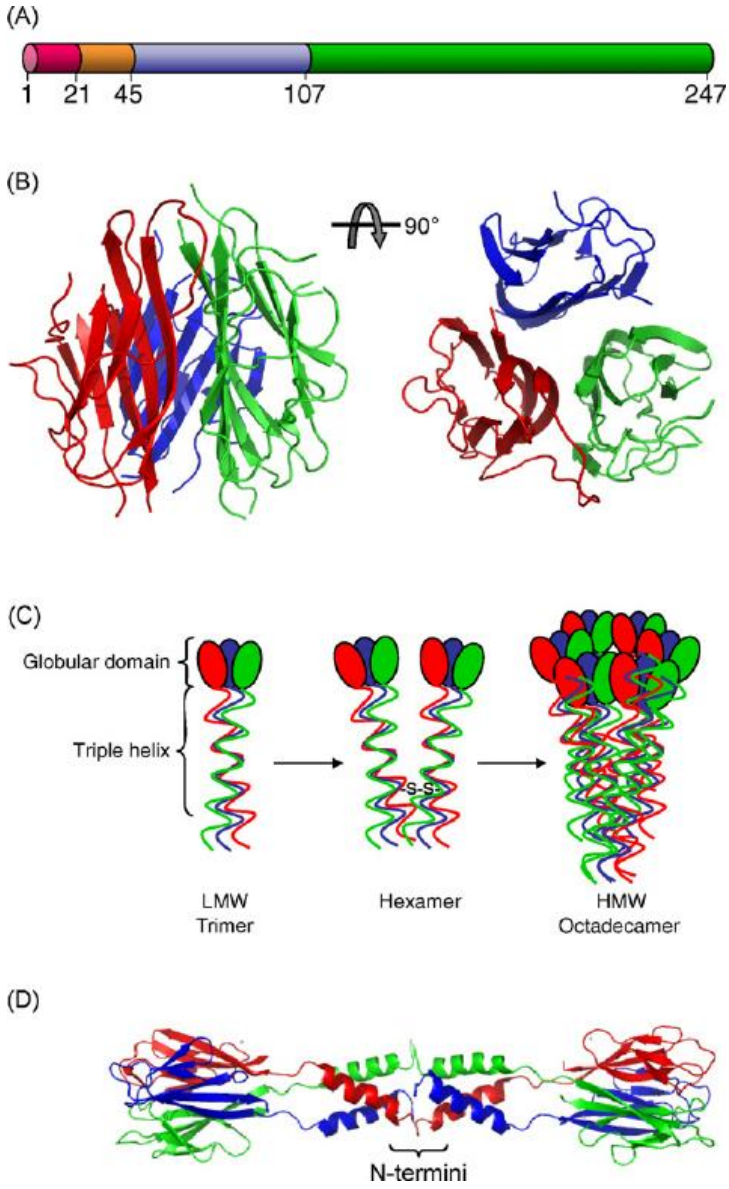

Figure 1. (A) Adiponectin monomers contain 247 amino acids, with a lengthy globular C-terminal domain. A collagen-like helical domain (amino acids 45-107) allows helices to combine via disulfide bonds to create different isoforms. (B) Secondary structure of adiponectin. (C) Adiponectin multimerization. (D) Collagen-like domain interactions in multimerization (Galic, Sandra & Oakhill, Jon & R. Steinberg, Gregory 2010).

Adiponectin monomers interact (see Figure 1) to form a low molecular weight isoform (LMW), which is a trimer. The trimers interact to form a medium molecular weight (MMW) isoform, which is a hexamer. Finally, the hexamers can interact a high molecular weight isoform (HMW) which is a 12-18mer (Wang and Scherer, 2016). These interactions result from post-translational modifications and intracellular assembly assisted by endoplasmic reticulum chaperones (Wang et al, 2008 and Figure 2). All of these isoforms

circulate in the blood. Overall, the HMW isoform is more effective in lowering glucose in studies of mice, and the ratio of HMW to total adiponectin has previously been used as a more accurate estimation of the glucose-lowering ability rather than simply the total adiponectin concentration (Wang and Scherer 2016).

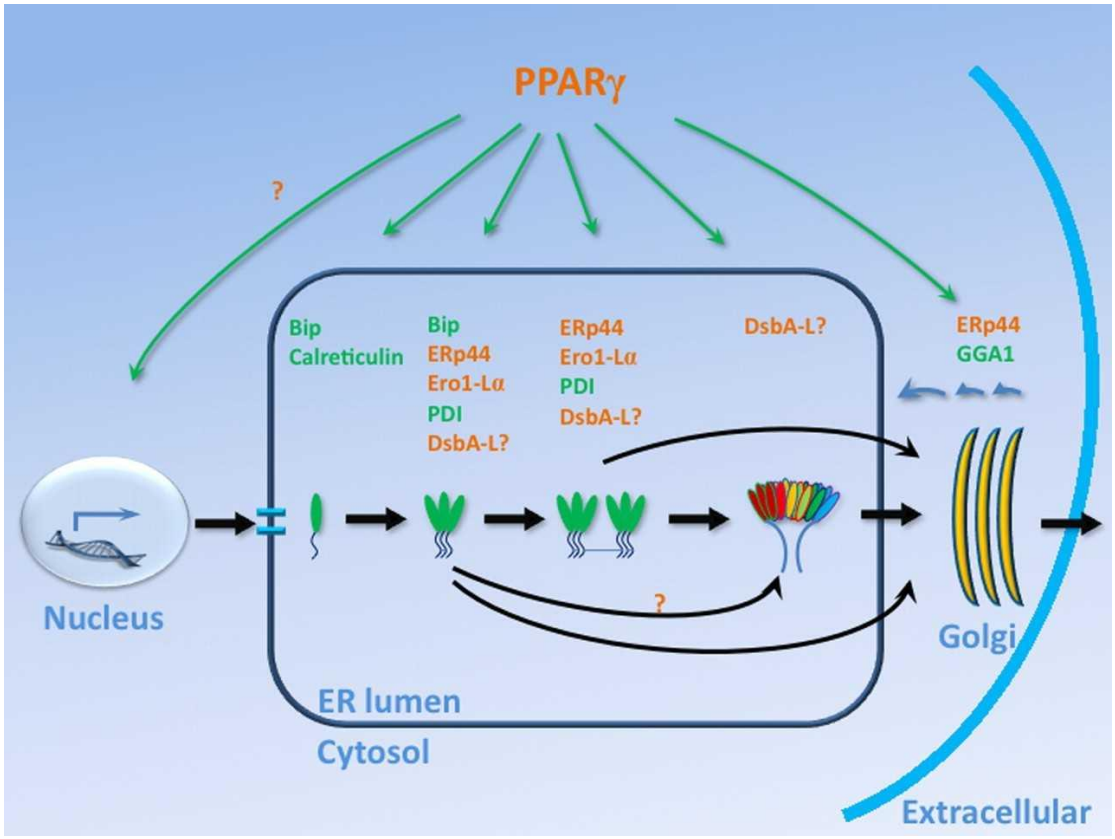

Figure 2 Adiponectin multimerization and control by chaperone proteins and the transcription factor PPARγ (Wang and Scherer 2008)

Surprisingly, plasma adiponectin has been shown to decrease in studies of obese mice (Nawrocki et al, 2005) and humans (Arita et al, 2012), and low levels of adiponectin are correlated with heightened insulin resistance and metabolic syndrome (Ruan and Dong 2016). Metabolic syndrome is defined as a condition where an individual possesses at least three of the five following traits: abdominal obesity, elevated blood triglyceride levels, low LDL cholesterol, high systolic or diastolic blood pressure, high fasting glucose levels (https://www.heart.org/en/health-topics/metabolic-syndrome/about-metabolic-syndrome

Adiponectin also lowers blood sugar by dramatically decreasing gluconeogenesis in the liver by inhibiting genes coding for enzymes involved in glucose production (Ruan and Dong, 2016 and Yamauchi et al, 2002) and increasing the utilization of glucose (Yamauchi et al, 2002). Specifically, adiponectin inhibits expression of phosphoenolpyruvate carboxykinase and glucose-6-phosphotase in hepatocytes through the activation of AMP-activated protein kinase (AMPK). These enzymes are necessary to perform gluconeogenesis (Ruan and Dong, 2016; Yamauchi et al, 2002 and Combs et al 2001), so the decrease of the activity of these enzymes reduces glucose output into the blood from the liver. Increased utilization of glucose caused by the activation of AMPK by adiponectin can also contribute to lowering blood glucose (Ruan and Dong, 2016). AMPK has been shown to increase fatty-acid oxidation and stimulate glucose uptake (Ruan and Dong, 2016). In addition to these mechanisms by which adiponectin improves blood glucose homeostasis and reduces the risk of hyperglycemia, adiponectin also has been shown to have other health effects such as suppressing cell death, suppressing hepatic lipogenesis, increasing macrophage proliferation, and reducing inflammation. (Ruan and Dong, 2016).

Certain receptors, Peroxisome Proliferator-Activated Receptors (PPAR), have been proposed to play roles in obesity and diabetes (Gilde et al, 2006). There are three different isoforms of PPAR: PPARα, PPARβ, and PPARγ. PPARα is common in tissues that tend to have more oxidative processes, like the liver and heart (Gilde et al, 2006). Fibrates that activate PPARα have been used to improve plasma lipid concentrations by lowering triglycerides and low-density lipoprotein (LDL) cholesterol and increasing high-density lipoprotein (HDL) cholesterol through multiple mechanisms (Gilde et al, 2006). These PPARα-activating fibrates also increase the oxidation of fatty acids by increasing the expression of genes that metabolize fatty-acids in oxidative tissues (Gilde et al, 2006). Through these and other

mechanisms, PPARα agonists and activators have been shown to decrease the risk of cardiovascular disease (Gilde et al, 2006). There has been less research done on PPARβ as it is expressed in all tissues, which makes its effects on specific tissues more difficult to measure (Gilde et al, 2006). Similar to PPARα, it has been shown to increase HDL and fatty acid oxidation in the heart and skeletal muscles (Gilde et al, 2006). It also decreases VLDL cholesterol. PPARγ is largely expressed and activated in white adipose tissue (Gilde et al, 2006) by translocation of PPARγ from the cytoplasm to the nucleus under the regulation of specific ligands. Ligands of PPARγ called thiazolidinediones (TZDs) are frequently used clinically to promote insulin sensitization in patients with type 2 diabetes by increasing the activity of PPARγ in adipose tissue. This has been reported to improve insulin sensitization in hepatic and skeletal muscle tissue (Gilde et al, 2006). PPARγ ligands have been suggested to promote adiponectin synthesis and multimerization (see Figure 2) in adipose tissue (Bodles et al, 2006 and Nawrocki et al, 2005), and adiponectin mediates the insulin-sensitizing actions of these ligands (Nawrocki et al, 2005), while obesity is associated with reduced circulating adiponectin (Wang and Scherer, 2016). Adiponectin regulates metabolism through PPARγ control of proteins promoting glucose and fatty acid uptake and oxidation (Ruan and Dong 2016).

As PPARγ ligands promote adiponectin synthesis, and adiponectin levels are decreased in mice who are obese, the hypothesis underlying this research is that PPARγ in the nucleus will be depressed in the white adipose tissue of the mice who have been fed a high-fat diet as a decrease in PPARγ could lead to a potential mechanism of the decrease of adiponectin in obese mice. Additionally, since HMW adiponectin has a larger effect on metabolism and insulin sensitization than either LMW or MMW, it is hypothesized that mice who have been fed a high-fat diet will have lower levels of HMW adiponectin in adipose

tissue and a lower HMW to total adiponectin ratio in adipose tissue as compared to mice fed a low-fat diet (Wang and Scherer, 2016). Such findings would support the hypothesis that PPARγ regulates the multimerization of adiponectin and can thus modify the ratio of HMW adiponectin to total adiponectin levels.

Methods

Animals. For this study, adipose tissue from male (strain C57BL/6J) mice initially weighing 20-25g and 8 weeks of age was obtained from Dr. Brad Hill at the University of Louisville. The mice from which the adipose samples had been obtained had been fed either a high-fat (60% fat/ 20% carbohydrate/ 20% protein) or a low-fat (10% fat/ 70% carbohydrate/ 20% protein) isocaloric diets from Research Diets, Inc. (New Brunswick, NJ) for 6, 10, or 16 weeks.

Sample Preparation for PPARγ Assay. Four samples of white adipose tissue were assayed for PPARγ from each group of mice from each of the three time periods. The samples were prepared using the Nuclear Extraction Kit from Cayman Chemical Company (Ann Arbor, MI) as described in the product insert. Briefly, samples (100-200mg) were homogenized with a plastic pestle operated with a hand-held motor using 3mL ice-cold 1X Complete Hypotonic Buffer with DTT and NP-40 per gram of adipose tissue. After 15 minutes on ice, the suspension was centrifuged at 300 × g for 10 minutes at 4° C. The supernatant was discarded, and 500 µL of ice-cold complete Hypotonic Buffer was added to the pellet for a more complete cellular lysis and mixed by pipetting up and down. Then the suspension was incubated on ice for another 15 minutes followed by the addition of 50 µL of 10% NP-40. The samples were centrifuged at 1400 × g for 30 seconds at 4° C. The supernatant was removed and discarded. The pellet containing intact nuclei (and the active fraction of PPARγ) was resuspended in 100 µL of ice-cold Complete Nuclear Extraction Buffer to extract PPARγ. The microcentrifuge tubes were vortexed for 15 seconds followed by 15 minutes of rocking on ice. They were then vortexed for 30 seconds followed by another 15 minutes of rocking on ice. The samples were then centrifuged at 1400 × g for 10 minutes at 4° C. The supernatant containing the extracted nuclear fraction was transferred to new

microcentrifuge tubes and stored at -80° C until used in the enzyme-linked immunosorbent assay (ELISA).

ELISA for PPARγ. After the samples were thawed, the amount of PPARγ in the samples was determined using an ELISA as described in the insert for the PPARγ Transcription Factor Assay Kit from the Cayman Chemical Company (Ann Arbor, MI). Blank wells, non-specific binding wells, along with a Positive Control Sample were run in duplicate along with samples containing 70µL Complete Transcription Factor Binding Assay Buffer (CTFB) and 30µL of the sample itself. After incubation, the wells were washed 5 times with the 1X Wash Buffer, and the primary antibody was added to all wells except for the blanks. After incubation, all wells were washed 5 more times, the secondary antibody was added to all wells excluding the blanks, and the plate was covered and incubated for 1 hour at room temperature. The plate was washed an additional 5 times, and the developer solution was added to all wells. After 45 minutes of incubation at room temperature while rocking, stop solution was added to all wells and the absorbances of the wells were read at 450nm using an EL_x808 microplate reader.

Tissue Preparation for Adiponectin Samples. White adipose samples were obtained from the same experimental mice for adiponectin analysis. Three samples from each group of mice from each of the three time periods were homogenized in an ice-cold lysis buffer containing 50 mM Hepes (pH 7.6), 150 mM sodium chloride, 1% NP-40, 5 mM ethylenediaminetetraacetic acid (EDTA), 1 mM sodium fluoride, 1 mM potassium pyrophosphate, 1 mM sodium orthovanadate, 5 µg/mL Alfa Aesar Protease Inhibitor Cocktail I (Haverhill, MA), and 1 mM phenylmethylsulphonyl fluoride. This was then

centrifuged at 10,000 × g at 4°C for 10 minutes. The supernatant was stored until use at -80°C

ELISA for HMW and Total Adiponectin. The ELISA for the HMW adiponectin and total adiponectin (ALPCO, Salem, NH) was performed using the method described in the product insert but modified for use on tissue extracts rather than on plasma. The ELISA allows the measurement of both total and HMW adiponectin by pretreating a portion of the sample with a protease (chymotrypsin) that selectively degrades the MMW and LMW adiponectin isoforms (Ebinuma and Masanao, 2009). The ELISA for plasma adiponectin was modified by adapting the method of Harris et al (2011), which was a modification of the plasma ELISA applied to the original medium of cell cultures. 50µL tissue extract was combined with 100µL of protease buffer and 100µL pretreatment buffer and incubated for 20 minutes at 37°C. Then, 40µL of both pretreated samples and untreated samples were combined with 1 mL of Dilution buffer. This brings the final dilution to 1:130. 50µL of the diluted solution was added to each well and the ELISA was performed as described in the product insert. The plate was covered and incubated for 60 minutes at room temperature. The plate was washed twice using wash buffer and 50 µL of Biotin conjugated primary antibody was added to each well and incubated for another 60 minutes at room temperature. The plate was washed three more times and 50 µL of the enzyme-labeled Streptavidin added to each washed well and the plate was covered and incubated for 30 min at room temperature. The plate was washed three more times and 50 µL of the working Substrate Solution was added. The plate was covered and incubated for 10 minutes at room temperature before 50µL of the Stop Solution was added to each well. The absorbance was read at a wavelength of 492 nm using an EL_x808 microplate reader. The quantitative value of HMW adiponectin and

total adiponectin was then able to be calculated using the standards provided and the dilution factor of 1:130.

Data Analysis for PPARγ: Absorbance values were multiplied by 1000 to calculate Absorbance Units (AU). Means ± standard errors were calculated for each group of mice. An ANOVA was performed to compare the low-fat values across time and high-fat values across time. A t-test assuming unequal variances was performed to make comparisons as necessary.

Data Analysis for High Molecular Weight and Total Adiponectin. Means ± standard errors were calculated for each group of mice. An ANOVA was performed to compare the total and HMW adiponectin in each of the timeframes. A t-test assuming unequal variances was performed to make comparisons as necessary.

Results

In this experiment, mice fed a either a high-fat diet (HFD) or a low-fat diet (LFD) were utilized. Cummings et al (2012), experimented on these same mice and reported on their body weights in the first six weeks and their food and water intake as shown below in figure 3. Body weights for the remainder of the 16 week feeding period were not available. HFD mice weighed more than those on the LFD despite no difference between food and water intake per kilogram body weight.

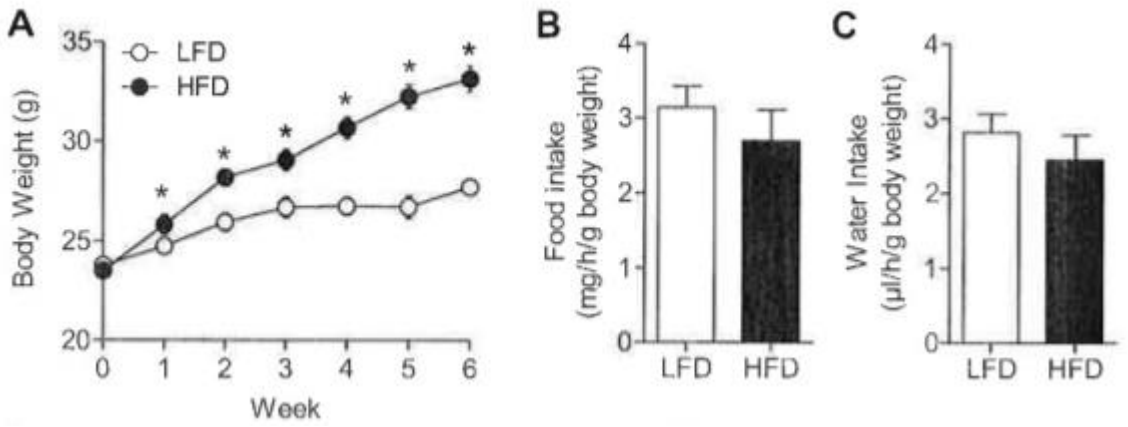

Figure 3. Body weight (A), food intake (B), water intake (C) in high-fat fed (HFD) and low-fat fed (LFD) mice. (Cummings et al, 2012). Mean ± standard error

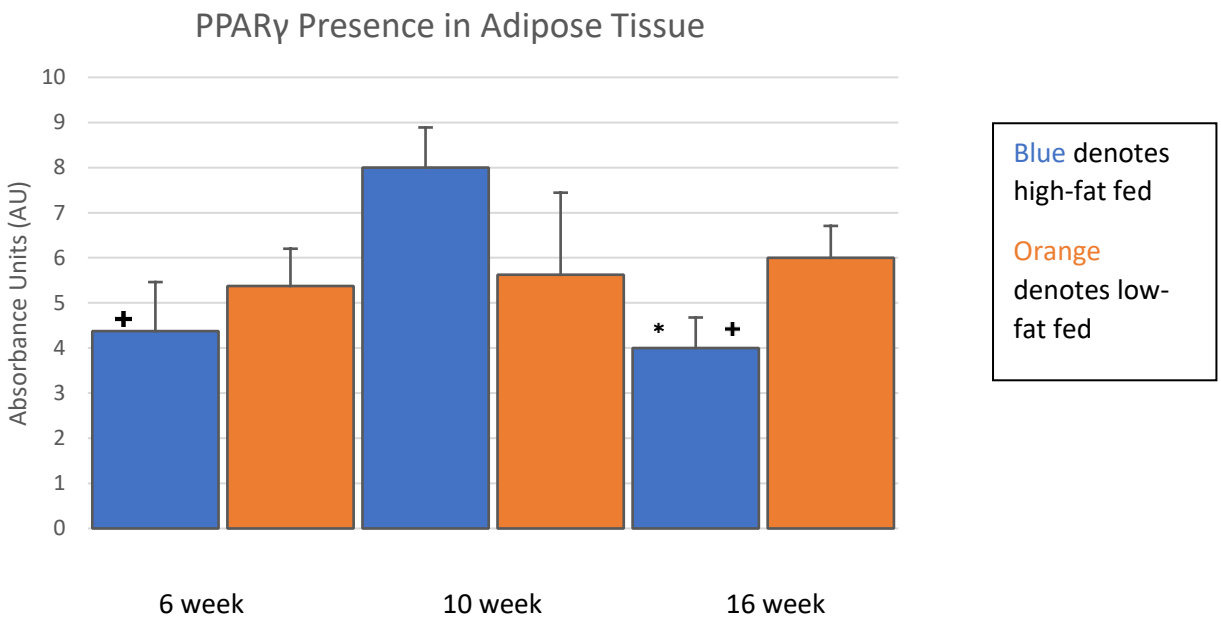

*Figure 4. PPARγ in adipose tissue HFD and LFD mice of all three time periods. The error bars shown reflect the standard error of the mean of the group. *= significantly (P=<0.05) different from 16 week LF value. += significantly different (P=<0.05) from 10 week HFD.*

Figure 4 shows the PPARγ values in each group over the course of the experiment. An ANOVA indicated there were no significant differences across time in PPARγ levels for the LFD group. However, ANOVA indicated a significant difference ($P<0.05$) in the HFD group, and a t-test indicated that the 10 week HFD group had significantly ($P<0.05$) greater PPARγ levels (100% increase) than either the 6 week or 16 week HFD groups. There were no statistically significant differences in the levels of PPARγ between HFD and LFD groups after 6 or 10 weeks of dietary manipulation. Between the 16 week groups, the HFD group was found to be significantly different ($P<0.05$) than the LFD group (approximately 30% decrease).

Because the composition of adipose tissue cells is predominately lipid, it stands to reason that normalizing results by expressing results relative to the amount of protein in the samples would be inappropriate. Therefore, it was the mass of total tissue utilized that was compared between each group to assure that variance between masses was not a factor in the comparisons illustrated in figure 4. An ANVOA was performed and indicated that there

were no statistically significant differences between the amounts of sample used among the 6, 10, 16 week groups. T-tests were performed to analyze differences between the HFD and LFD groups at each time period. There was a statistically significant difference in the amount of sample used between the HFD and LFD samples for the 6 week group ($P<0.05$) but the larger average mass of samples used for the HFD mice did not yield a statistically significant increase in the nuclear concentration of PPARγ. In fact, the value for the 6 week HFD mice was slightly, though not significantly, lower than that of the 6 week LFD mice.

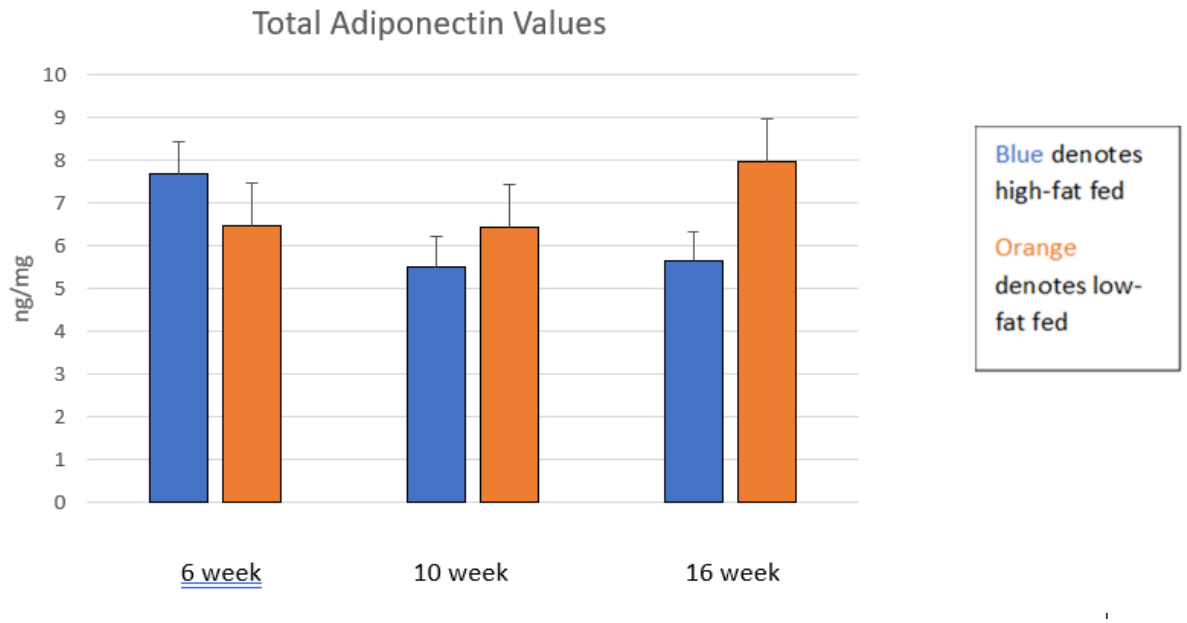

Figure 5. Total adiponectin (ng/mg tissue) in high-fat fed and low-fat fed mice of all three timeframes. The error bars shown represent the standard error for each group.

The standard curve for adiponectin yielded a correlation coefficient of 0.994 indicating a linear fit of the data. In Figure 5, the average amount of total adiponectin in each of the groups of mice is shown in nanograms of adiponectin per milligram of fat tissue. Analysis by ANOVA indicated that there was no significant difference in total adiponectin levels across the LFD groups, despite the appearance of a small increase over time. Likewise, ANOVA indicated no significant differences across time for the HFD groups over time, despite the

appearance of a decrease. There were no significant differences between HFD groups and LFD groups at any of the timepoints of the experiment.

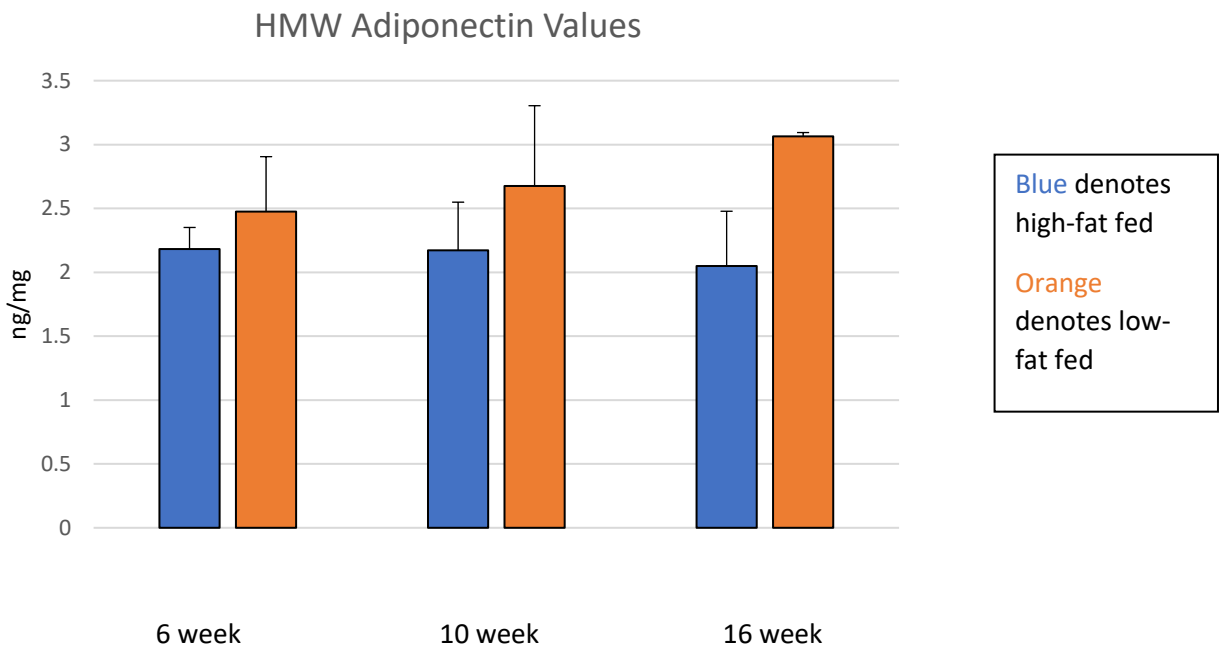

Figure 6. High molecular weight (HMW) adiponectin (ng/mg tissue) in each group of mice . The error bars denote the standard error for each group.

In Figure 6, the average amount of HMW adiponectin in each of the groups of mice is shown in nanograms of adiponectin per milligram of fat tissue. Analysis by ANOVA indicated that there were no significant differences in HMW adiponectin levels across the LFD groups, despite the appearance of a small increase overtime. Likewise, ANOVA indicated no significant differences across time for the HFD groups over time, despite the appearance of a decrease. There were no significant differences between HFD and LFD groups at any of the timepoints of the experiment.

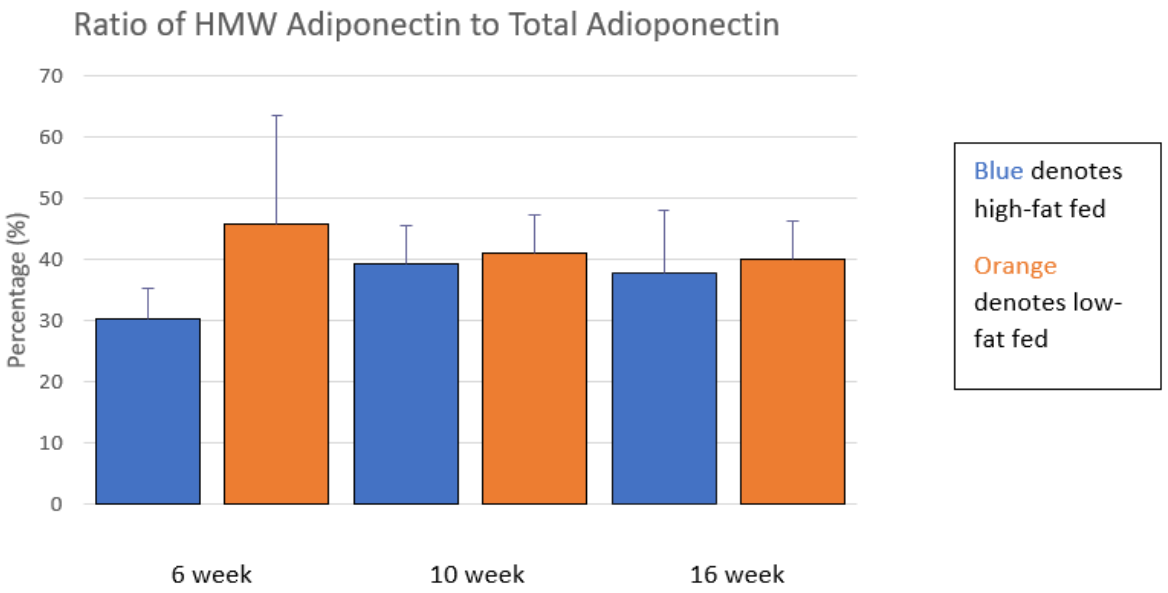

Figure 7. The ratio of HMW adiponectin to total adiponectin.

In Figure 7, the ratio of HMW adiponectin to total adiponectin is given as percentages. Analysis by ANOVA indicated that there was no significant difference in total adiponectin that is represented by the HMW isoform ratio across the LFD groups. Likewise, ANOVA indicated no significant differences across time for the HFD groups over time. There were no significant differences between HFD and LFD groups at any of the timepoints of the experiment.

Discussion

The correlation between adiponectin and obesity has been well-documented throughout the past two decades. Studies have shown that circulating adiponectin levels are lower in both mice (Nawrocki et al, 2005) and humans (Arita et al, 2012) who experience obesity. There is also evidence that the decrease in adiponectin with obesity is primarily due to the decrease in HMW adiponectin and that the ratio of HMW adiponectin to total adiponectin is more important to the control of insulin sensitization. Since the HMW form of adiponectin has been demonstrated to be most effective at supporting the activity of insulin, any mechanism should be able to account for the specific effects on this isoform as well as the total concentration circulating in the blood. This work is focused on clarifying the potential role of PPARγ as the mechanism underlying the adiponectin effects during obesity.

There are multiple models utilized in the study of obesity (Lutz and Woods, 2012). These can broadly be considered as either genetic or nutritional models. Prominent genetic models include the ob/ob and db/db mice with genetic defects in leptin production or detection and the fa/fa Zucker rat which involves a mutated leptin receptor. Nutritional models include cafeteria diet-induced obesity which mimics the so-called Western diet of humans and high-fat diet obesity. In the present experiment groups of mice were given either a control diet (low-fat) or a high-fat diet, for 6, 12, or 16 weeks as a model for obesity. Figure 3 indicates that there is a significant difference between the weights of mice on the different diets within the first six weeks of feeding, suggesting that this technique of inducing obesity is effective. Tissues from mice utilized in this study were obtained as an additional project from another laboratory. Information regarding the subsequent body weights was not able to be obtained from this laboratory. However, it was assumed that

these differences were maintained or increased throughout the time course of the experiment.

The PPARγ ligands rosiglitazone and pioglitazone (TZDs) promote insulin sensitization and are clinically utilized for this effect in humans (Gilde et al, 2006). Ligands of PPARγ have been shown to have effects on adiponectin synthesis and multimerization in white adipose tissue (Bodles et al, 2006 and Nawrocki et al, 2005). These TZDs have been shown to increase insulin sensitivity through regulation of the expression of the mRNA for adiponectin (Maeda et al, 2001). Wang and Sherer (2008) have suggested the role of PPARγ in regulating the multimerization process through control of endoplasmic reticulum chaperone proteins (see Figure 2). PPARγ has been shown to regulate adiponectin in several different ways. It has also been suggested that PPARγ regulates proteins that promote glucose and fatty acid uptake and oxidation (Ruan and Dong 2016) which would further regulate blood glucose levels. With these multiple potential connections between PPARγ and adiponectin, this project has focused on the mechanistic role of PPARγ in the obesity-associated changes in adiponectin.

It was hypothesized that there would be a decrease in adipose tissue levels of PPARγ with obesity i.e. that is to say there would be a lower concentration of PPARγ in the nucleus of fat cells from HFD mice compared to LFD mice. Additionally, it was hypothesized that the longer the mice had been on the diet, the greater the difference between the HFD and LFD groups. The results for PPARγ indicated some significant effects of diet. While PPARγ was not significantly different between 6 week and 10 week HFD and LFD groups, it was reduced after 16 weeks of high-fat feeding. Therefore, these data from the 16 week HFD group support the hypothesis, but the effect seems to be quite delayed. There was no statistically significant difference in the amount of PPARγ in the HFD and LFD mice at the 6 week time

point, though there was a tendency for slight decrease in PPARγ in the HFD mice. This is relevant because, despite the lack of significant difference in the amount of PPARγ, a significantly greater amount of fat was utilized ($P<0.05$) from the 6 week HFD mice from the LFD group. This suggests that some effects of PPARγ may have been detected as early as 6 weeks of diet manipulation and supports the hypothesis of adipose tissue PPARγ changes in obesity.

Studies have shown that circulating adiponectin levels are lower in both mice (Nawrocki et al, 2005) and humans (Arita et al, 2012), who experience obesity. There is also evidence that the ratio of HMW adiponectin to total adiponectin is more important than total adiponectin levels regarding insulin sensitivity (Wang and Scherer 2016). It was hypothesized that there would be a significant reduction in total adiponectin and HMW adiponectin in the adipose tissue samples from the HFD group compared to the LFD group and that the differences between the two groups would be accentuated by time on the two diets. It was also hypothesized that the length of exposure to the high-fat diet, the smaller the ratio between the HMW adiponectin to total adiponectin.

There was no statistically significant difference in total adiponectin (nanograms of adiponectin per milligram of adipose tissue) between HFD mice and LFD mice at any of the three time points (Figure 5). The finding that adipose tissue total adiponectin levels was unaffected does not support the original hypothesis. Previous studies indicating a significant decrease in adiponectin in obese subjects may still be consistent with the data from the current study (Nawrocki et al, 2005 and Arita et al, 2012). Circulating levels of adiponectin might not only be related to levels of adiponectin synthesis in adipose tissue but could also be related to the rate of secretion from the tissue or alteration in the half-life of circulating adiponectin. Additionally, there were no significant differences (Figure 6) in

the levels of HMW adiponectin (nanograms of adiponectin per milligrams of adipose tissue) nor the ratio between HMW adiponectin and total adiponectin (Figure 7). Despite the lack of statistical significance in the adiponectin data this could be a reflection of the small number of tissue samples analyzed (3 per group). There certainly are indications in Figures 5 and 6 that difference between the groups, while not statistically significant, were becoming apparent. Additional experiments which increase the number of samples analyzed could lead to a finding of statistical significance.

Research regarding adiponectin and possible ligands that control its production and multimerization have a variety of different possible clinical applications regarding cardiovascular disease, diabetes, atherosclerosis, stroke and complications that occur with these conditions. In the model of obesity used in this study, PPARγ levels appear to be reduced only after a lengthy duration of high-fat feeding. This may still indicate some degree of mechanistic regulation of physiological and biochemical changes during obesity by PPARγ. However, since neither total nor HMW adiponectin were significantly affected in this model of obesity, there does not seem to be a strong correlation between changes in PPARγ and total adiponectin or isoform multimerization. Further work may need to explore other roles for PPARγ or other mechanisms regulating insulin sensitivity.

The results of this study do not agree well with the hypotheses outlined in the Introduction. This may result from several limitations which are often inherent when studying a complex topic such as obesity, where the disease is potentially caused by a spectrum of interacting factors. One limitation unique to this study has been mentioned above, a small sample size for measurements of both adiponectin and PPARγ. In addition, the results of this type of study are constrained by the diets available, which do not exactly mimic the great diversity of foods available in a diet that may contain items freely

chosen. In addition, the mice in both experimental treatments are similar quantities of food, which is not always consistently observed between lean and obese individuals. Furthermore, while the HFD group in this study received 60% of calories from fat, the type of fat ingested may have a significant influence on the response. For example, the currently popular "Mediterranean diet" includes high quantities of fat but is not associated with obesity. A number of these factors should be part of future research on the topic of the adiponectin and PPARγ relationship to obesity.

Works Cited

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